

On *Pieris (Artogeia) marginalis macdunnoughii* Remington (Pieridae)

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Abstract. Some previous work on *Pieris (Artogeia) ssp. macdunnoughii* and *ssp. marginalis* is summarised. Experiments in which Colorado *macdunnoughii* were hybridized with European *P. napi* L. are reported, with particular attention to inheritance of yellow pigmentation and viability of F_1 hybrids: no females are obtained from *napi* ♀ × *macdunnoughii* ♂. *Macdunnoughii* carries two distinct systems of yellow coloration, both the bisexual recessive sulphurea and the female-limited dominant flava. Whether this unusual condition has any ecological or evolutionary significance is uncertain. It is concluded that neither subspecies *marginalis* nor *macdunnoughii* is conspecific with the typical *napi* of Europe, but Eitschberger's opinion that *macdunnoughii* is a subspecies of a species *marginalis* Scudder may well be correct. However, the relative position of the subspecific taxa *venosa* Scudder, *microstriata* Comstock and *mogollon* Burdick requires experimental investigation.

Introduction

This Colorado subspecies was described as of *Pieris napi* L. by Barnes & McDunnough in 1916 under the name *pseudonapi*. The type-locality was "Silverton, 10,000 ft., where it is single-brooded". The description as very close to European *napi* "although in the females the black dots of the primaries are practically obsolete" is very meagre for a subspecies; the females varied in the amount of black markings. The completeness of the picture is improved if we can add the authors' description of *pallidissima*, the second generation at Provo, Utah, which they seem to have regarded as consubspecific with *pseudonapi*.

Remington (1954) pointed out that the name *pseudonapi* had been used by Verity (1911) for a race of *Pieris melete* Ménétrières, and re-named the Colorado insect *P. napi macdunnoughii*. He added that the series of *Pieris napi* at Yale University from Colorado, Utah, Nevada and Wyoming [i.e. the populations considered as subspecies *macdunnoughii* and *ssp. pallidissima* by Eitschberger 1983] indicate that all represent a single race. He says, "My series from the Teton Mountains of Wyoming shows very dark and very pale individuals taken flying together" — one supposes that the reference is to melanic markings, not to any yellow pigmentation. I conclude that Remington, like Barnes & McDunnough before him, regards *macdunnoughii* and *pallidissima* as showing no differentiation worthy of separate subspecific naming.

Eitschberger (1983) treats these populations as separate subspecies of a species *Pieris marginalis* Scudder, which is said to include also *mogollon* Burdick (New Mexico) and six newly named subspecies as well as ssp. *marginalis* (Washington Terr.) and perhaps *hulda* Edwards (Alaska).

Warren (1963), working from androconial scales, had attributed ssp. *marginalis* to *napi* and ssp. *macdunnoughii* to a species *oleracea* Harris, but the present writer is inclined to follow Eitschberger in respect of these two subspecies, if only on account of their special pigmentation.

The Ssp. *marginalis*

In 1970 I reported experiments in which a N. W. Oregon (Saddle Mt. State Park) stock of "*Pieris napi marginalis*" was crossed with the bright yellow British *napi* form *sulphurea* Schöyen (Head's "citronia") and with certain other European forms. The Oregon insects were yellow in ground-color: very faintly citron-shaded in the males but deeper in the females. The markings of the latter sex differed from those of *napi* by the often unequal development of the melanic markings: though the forewing hind-marginal streak was generally present, the second and even more the first discal spots were much reduced and often absent.

The breeding results seemed to indicate that the population was homozygous for an intermediately recessive gene of the subalbasulphurea series (Bowden 1963), indistinguishable from that producing "Thompson's pale yellow" rarely in the British Isles. The conclusion then was that "pale yellow" heterozygotes should be sought in other American subspecies of the *napi* group: it was hardly expected that other homozygous populations would be found, but *macdunnoughii* might perhaps be polymorphic in respect of *sulphurea*?

However, the flava (ochreous) color which was found in the F_1 *napi*-hybrid females was then attributed to the European parents and it was not realized that the *marginalis* stock might also carry some flava. But Shapiro (1985, *in litt.*) had noticed that some *marginalis* populations had high frequencies of a buff female. Looking at the 1970 *marginalis* specimens now, it seems beyond dispute that the females possess an ochreous tinge as well as the lemon, and that the F_1 hybrid females' color is unlikely to have come entirely from the European side.

The Ssp. *macdunnoughii*

In 1971 I reared only two females of this subspecies from Gunnison Co., Colorado (W.B. Watt). They were caged with two (later four) English (Herts.) males, but these paid no attention, though brothers were pairing with other butterflies in nearby cages. After two days three (later five) Scottish males were supplied, but again showed no concern. A male from French Pyrenees showed minimum interest. It appeared that at least these two individual females had no pheromone attraction for European males.

Not until 1985 were we able to raise *macdunnoughii* in adequate numbers (brood 1985-*r*), by pairing offspring of females taken in Grand Co., Colorado, in 1984 (Shapiro). We found that the fresh females were definitely a greenish yellow (very near the color of the forewings of *Gonepteryx rhamni* L. ♀), the males very palely yellow, but quite distinct from the white of *napi*. Apart from slight forewing apical blackening, our males were typically without marking, and the females carried only the forewing discal spot that normally marks *napi* males; even this was sometimes absent and the hind-marginal female streak hardly ever showed at all.

Thus comparing the markings of these two subspecies with those of typical *napi*, the females departed from type in opposite directions: the expression of *marginalis* forewing markings was biased posteriorly, those of *macdunnoughii* even diminished in that direction.

Experimental

Macdunnoughii males of brood 1985-*r* paired in August 1985 with British *napi* f. *sulphurea* females, but the mere 15 eggs produced only nine pupae, of which six (presumably female) proved unable to develop. One male hybrid emerged normally in September, a very pale lemon yellow, with faint black forewing apices and spots, and very slight veining below. Two more pupae colored as males and split their thoraces but failed to eclose: these were dissected out and appeared similar to the first male. This rather disappointing result, as far as it goes, does indicate that the *macdunnoughii* lemon tint, like that of *marginalis*, belongs to the *sulphurea* series of alleles.

Three other pairings of *macdunnoughii* males (one from 1985-*r*, two from the subsequent inbred 1985-*q*) were made with single *napi* females of mainly European stock (which however carried some genes derived from ssp. *oleracea* and were of *funebis* form — see Lorković 1971, Bowden 1983). The resulting broods (1985-*k*, 1986-*g*ⁱ, *g*ⁱⁱ) were large, but nevertheless only male hybrids emerged, even after diapause; their upperside ground-color was uniformly pure white, without yellow toning, as expected from heterozygous *sulphurea*. There was indeed one female in *g*ⁱⁱ, but this was rejected. (*Marginalis* and *macdunnoughii* hybrids with *napi* usually carry radiating black markings on the distal ends of the veins. Such are uncommon on pure ssp. *napi*, and *napi* waifs can thus be recognised, though without certainty.) The melanic pigmentation of the hybrids may be described and discussed later.

The apparently more difficult reciprocal hybridization was obtained in August 1986, using a *funebis* heterozygote male of 1985-*h*. The *macdunnoughii* female came from brood 1985-*q*. It was expected that in this case female hybrids would precede the males, if the apparent sexual imbalance in the earlier hybrids was due to a disturbance of diapause control. In this brood, 1986-*n*, losses of larvae in early stages were appreciable, probably 15-20%. Twenty males emerged 14-21.ix.86,

followed by a mixture of 19 ♂ + 25 ♀ by 23.x.86, giving a final ratio of 8 ♂ : 5 ♀. A few other pupae died.

Surprisingly, all the F_1 hybrid females were of a light yellow color, rather with a flava tinge, and none were very near white. Thus this color was inherited from the *macdunnoughii* mother in the dominant mode, and was expressed only in the females.

It was thought unlikely that a straight F_2 hybrid brood would be productive. Males of the first F_1 , 1985-*k*, were back-crossed in each direction:

♀♀ funebris hz., 1985-*h* × ♂♂ 1985-*k* → 1986-*c*, 33 ♂ + 24 ♀,

♀ *macdunnoughii*, 1985-*r* × ♂ 1985-*k* → 1986-*j*, 5 ♂ + 3 ♀,

the sexes here being not significantly far from equality ($\chi^2 = 1.4$, $p = 0.23$ and $\chi^2 = 0.5$, $p = .90$ respectively). Brood 1986-*c* gave 57 adults the same year. All the males were white, as were some of the females, others varying from slightly tinted up to near a full flava color in two cases. The pigmentation here was definitely not of the sulphurea series, and as the brood had two mothers, a ratio 2 flava: 22 no-flava would be meaningless. Fifteen individuals of 1986-*c* were funebris (though of varying expression), almost exactly the anticipated number.

The other back-cross 1986-*j* resembled pure *macdunnoughii* in respect of wing ground-color: tinted citron even in the males, very definitely so in two of them. The one white male was small (2×19 mm), but the other seven insects were large. The striking markings of several females, possibly influenced by the funebris gene, may be discussed in a later paper.

Pigmentation Systems

More than thirty years ago (1954) I was able to write of general agreement that there were at least two distinct forms of *napi* with extra yellow on the wings: ochreous flava Kane confined to the female and with the color *not* extending to the forewing disc underside, and the primrose sulphurea Schöyen with the more extensive bisexual coloration. Thus all the *Pieris* butterflies with yellow wing-uppersides have since been interpreted in terms of these two modes:

female only — brownish — dominant or semi-dominant genetically
bisexual — citron yellow — recessive genetically.

So the tests are:

if a problem male (white or yellow) crossed with a sulphurea female *napi* produces any yellow sons, it carries sulphurea;

if a problem female (yellow or ochreous) crossed with a sulphurea male *napi* produces any yellow sons, it carries sulphurea; if only white sons, it carries flava;

if a problem female crossed with wild-type *napi* produces any yellow daughters, it carries flava,

provided that numbers are adequate.

In both *flava* and *sulphurea* the saturation of the color can vary from very faint to intense, sometimes by the action of different alleles (cf. Bowden 1961) and sometimes environmentally. Some *sulphurea* alleles give only a *very* faint coloration to male butterflies: in all doubtful cases the forewing underside disc should be examined (Bowden 1961). Also, the *sulphurea* color is particularly liable to fade, especially in sunlight. At paler levels visual discrimination between *flava* and *sulphurea* becomes more uncertain.

It was known that both kinds of color could occur together in the same individual — Bowden (1962) described the transference of *sulphurea* genes to *P. (napi) bryoniae* Ochseneimer. Unfortunately I never considered that any natural population might constantly use a combination of the two systems of pigmentation. It was thus that I came partly to misinterpret the *Oregon marginalis* coloration.

When the pigments do co-exist in uncertain proportions in the females of a species they are difficult to distinguish visually, even in a fresh specimen. Photographic rendering is usually imperfect. If the *flava* and *sulphurea* systems are acting together in a population with yellow males, it is possible to identify them by hybridizing with *napi* carrying neither *flava* nor *sulphurea*. The result in the F_1 should be white males plus ochre-tinted females. This is the result obtained with both *marginalis* and *macdunnoughii*, which therefore carry *flava* as well as *sulphurea*.

Is the matter therefore to be considered closed? Perhaps not quite. Though *sulphurea* and *flava* pigments are generally separable by eye, chemical composition within each class may not be constant, and genetic controls in these Pierids may involve more than two loci.

Moreover, some biological aspects of the situation remain puzzling. Have these fainter colors *any* ecological effect? Do they offer clues to the phylogeny of taxa carrying them? Bowden (1977) figured a female *macdunnoughii* extremely reflective of U.V. light, indeed much more so than *marginalis*, but this phenomenon needs wider investigation in N.W. American *Artogeia*.

Specific Status

How distinct from *Pieris napi* L. is ssp. *macdunnoughii*? And how close to ssp. *marginalis*? The pterin pigmentation systems of these two subspecies are so similar as to suggest a close relationship. How do they react to hybridization with the European species?

Male *napi* have seemed rather unwilling to pair with *macdunnoughii* females, but one cage pairing occurred within a quarter of an hour. The reciprocal pairing is easy. In either case fertility is very good.

But the three large broods ♀ *napi* × ♂ *macdunnoughii* produced no F_1 daughters (the one female obtained showed no characters negating a pure *napi* origin and was probably a waif). The one brood 1986-*n* from

♀ *macdunnoughii* × ♂ *napi* yielded both sexes, but females were the less numerous (40%) and generally appreciably smaller; their fertility was not tested.

It is appropriate to compare the results for the *marginalis* × European hybrids reported in 1970:

napi ♀ × *marginalis* ♂ → 1968-b, 7 ♂ + 1 ♀ + 15 undeveloped pupae,
marginalis ♀ × *napi* ♂ → 1968-a, 9 ♂ + 8 ♀ (incl. 1 ♂ after diapause),
 Irish *napi* ♀ × *marg.* ♂ → 1966-i, 38 ♂ + 1 ♀ + 15 undeveloped pupae,
marginalis ♀ × Irish ♂ → 1968-j, 32 ♂ + 39 ♀.

They agree very well with those quoted above for *macdunnoughii*: on the whole it can be said for both ssp. *marginalis* and ssp. *macdunnoughii*, the cross ♀ *napi* × ♂ *m.* produces no viable female offspring — it is only too likely that the single females in 1968-b and 1966-i were waifs.

Such a sexual disturbance is more serious than a diapause disorganization, and is normally sufficient ground for a specific separation (cf. Lorković 1978). One could, however, expect *marginalis* and *macdunnoughii* to be mutually fully fertile and so probably conspecific. This should be confirmed experimentally, if possible.

Subspecific Relationships

It will be necessary also to study sexual relations between these subspecies and adjacent ones, especially *microstriata* Comstock and *venosa* Scudder. Shapiro (*in litt.* 1979) stated that *microstriata* seemed to intergrade into *marginalis* in N. W. California. Geographic boundaries between these taxa, as well as the other adjacent "subspecies" mentioned by Eitschberger (1983), may be very uncertain (even indefinite), and it can be unsafe for us to generalize from results obtained on constituent local demes. Differences may be in part environmental (e.g. in altitude) rather than genetic. It would be desirable to re-consider the criteria for separating subspecies in this area.

Our (1981) experiments with ssp. *microstriata* were incomplete, as acknowledged at the time, and there was a mistake in the report (p. 3, para. 5). This should read:

"One good female emerged on 20.v.79, and from this one individual all hybrid broods were derived. About 1-2 hr. after caging with a British sulphurea heterozygote *napi* male she paired with him . . ."

However the argument is little affected and the conclusions are unchanged. The female F₁ hybrids included pale yellows with the forewing underside disc pale yellow; a sulphurea allele must have been present in the white *microstriata* female used. This could be taken as suggesting at least introgression from *marginalis* or *macdunnoughii* into ssp. *microstriata*, which could conflict with Eitschberger's (1983) specific separation of *venosa* and *marginalis*.

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